EVALUATION OF *Panicum virgatum* L. FOR NITROGEN RELATED AGRONOMIC CHARACTERS

BY

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Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Chairman: Rex L. Smith
Major Department: Agronomy

Selected and non-selected half-sib progenies and their maternal parents of switchgrass, Panicum virgatum L. were evaluated for nitrogen related agronomic characters. The objectives were: 1) to evaluate associative nitrogen fixation and nitrogen use efficiency in switchgrass; 2) to evaluate switchgrass as a suitable forage crop for the state of Florida; and 3) to evaluate the potential for improvement through breeding.

In the field, over a two year period at two locations, significant variations were observed for yield, percent nitrogen, nitrogen fertilizer use efficiency and nitrogen fixation estimated by the total nitrogen difference method. Responses were influenced by maternal line origin, location, plant establishment and applied nitrogen fertilizer level. Broad-sense heritabilities for the field measured parameters were generally low, indicating that breeding progress will be slow. In the greenhouse, four populations of switchgrass showed significant differences in yield, nitrogen fertilizer use efficiency and nitrogen fixation estimated by total nitrogen difference and by nitrogen-15 dilution. Responses were

influenced by maternal line origin, population of selection and plant establishment.

Mean estimates of nitrogen fixation in the field by total nitrogen difference and acetylene reduction cores were 20.8 and 6.5 kgN/ha, respectively, and the two estimates were positively correlated ($P > 0.001$). Mean estimates of nitrogen fixation in the greenhouse by total nitrogen difference and nitrogen-15 dilution were 2.4 and 9.5 kgN/ha, respectively, but in two combined harvests, the estimates were negatively correlated ($P = 0.02$).

In all cases, %N in switchgrass was lower than in 'Pensacola' bahiagrass which was included as a check species. Dry metter yields were higher than in bahiagrass. This indicates that the ability of switchgrass to dilute nitrogen is a factor that makes it a promising forage and biomass producer, especially under low management and low nitrogen input conditions.

INTRODUCTION

Nitrogen is a vital element in the composition of plants and animals. Even though our atmosphere contains 78% nitrogen, it exists in the unavailable diatomic state. It is only after this diatomic species has been chemically reduced that it is rendered available for use in physiological systems. On a commercial scale, the reduction of nitrogen for use as applied fertilizer requires high energy inputs. With recent and justified concern over wise, efficient energy use, the world must look towards biological nitrogen fixation as an asset in food production.

Isolation of the diazotroph Azospirillum brasilense, from the rhizospheres of several tropical Gramineae in Brazil, sparked worldwide interest in the establishment of a highly efficient, associative nitrogen-fixing system that would augment global nitrogen inputs by the long and highly regarded legume-Rhizobia symbiotic systems. Subsequent research in Brazil, the United States, Great Britian and Australia repeatedly demonstrated yield increases due to inoculation with strains of A. brasilense in many tropical and temperate grass species. Confirmation of nitrogen fixation via acetylene reduction, ^{15}N and Kjeldahl nitrogen difference methods has been accomplished in ambient and axenic conditions; however, correlations to the observed plant responses have not been adequate. Alternate hypotheses for growth stimulation, e.g., production of plant growth regulators by bacterial inocula, have been proposed and demonstrated but these data

alone do not provide adequate explanations of observed responses. Other existing and introduced soil diazotrophs, alone and in combinations, continue to be studied in associative systems involving various grasses.

Advances in the understanding of associative biological nitrogen-fixing systems remain elusive, despite intensive research efforts of the past few years. Data relative to the magnitude and significance of these systems are usually empirical and center around visual observations of grass field crops and existing grassland ecosystems. Gross nitrogen recoveries and efficiencies continue to be highly encouraging; however, experimental data demonstrating nitrogen fixation *per se* are, for the most part, inconclusive. It would seem that either the individual systems themselves are highly variable and unpredictable or that measurement techniques are inadequate, or both.

This study is based on empirical observations of established, unfertilized high-yielding plots of switchgrass (*Panicum virgatum* L.) growing at the Agronomy Farm, University of Florida. The objectives are 1) to evaluate associative nitrogen fixation and nitrogen efficiency in switchgrass; 2) to evaluate switchgrass as a suitable forage crop for the state of Florida; and 3) to evaluate the potential for improvement through breeding.

REVIEW OF LITERATURE

Nitrogen Fixation

Huge amounts of nitrogen required for the growth of plants and animals are ultimately obtained from the atmosphere as chemically fixed forms. Fixation catalyzed by lightning and ultraviolet radiation may account for up to 0.5% of the global nitrogen requirement while man-made fertilizer nitrogen, requiring a large and costly energy input, accounts for an additional 5%. Biologically fixed nitrogen constitutes the remainder, estimated at 10^8 to 10^9 tons of N per year (54).

Biological nitrogen fixation occurs in a variety of situations under many different conditions. Plants belonging to the family Leguminoseae have evolved efficient symbiotic systems that fix significant amounts of nitrogen (27). These systems involve the formation of nodules, small compartments on the plant roots, that contain bacteroids of Rhizobium spp. Associative nitrogen fixation involving numerous bacteria of the family Enterobacteriaceae and grass crops is regarded as one of the most promising areas in biological nitrogen fixation research (45). Other documented biological nitrogen fixing systems include Klebsiella pneumoniae living in the intestines of humans on a high carbohydrate diet; various intestinal microflora in some termite species; Actinomycetes in nodule-type structures in the genera Alnus, Myrica, Ceanothus, Casuarina and Comptonia; and blue-green

bacteria growing as soil crusts, in aqueous solutions and in the rhizoids of plants of the genus Azolla (16,30,66,75).

Measurements of Nitrogen Fixation

Three basic techniques are available to measure nitrogen fixation. Total nitrogen present in the plant material can be measured by the Kjeldahl method, and after the subtraction of applied nitrogen sources, an estimate of nitrogen fixation may be obtained. Acetylene reduction techniques use the alternative nitrogenase enzyme substrate acetylene, which upon reduction produces ethylene that is measured by gas chromatography, and an estimate of nitrogen fixation may be calculated. Nitrogen-15 enrichment and dilution techniques estimate fixation by mass spectrometric measurements of $^{15}N_2$ (gas) incorporation in an enclosed system or the relative dilution of applied ^{15}N fertilizer in plant tissue by fixed nitrogen. All three methods have certain limitations and advantages.

Total nitrogen difference estimates by the Kjeldahl method (14) require extremely precise sampling, sub-sampling and analytical techniques. Serious errors may be introduced, especially in grass-bacterial systems, where there exists a large amount of soil nitrogen relative to fixed nitrogen, and the amounts of fixed nitrogen are on the same order of magnitude as the experimental error. Nonetheless, total nitrogen techniques have been widely used and provide gross estimates of nitrogen fixation and overall nitrogen relationships.

Due to its relative rapidity, simplicity and sensitivity of 10^3 to 10^4 times that of ^{15}N methods, the acetylene reduction technique is presently used in most nitrogen fixation studies (33). Calculations

include the assumptions that a theoretical reduction conversion ratio of $3C_2H_2 : 1N_2$ is maintained, non-interference by endogenous ethylene evolution and microflora capable of metabolizing acetylene and ethylene, and that the rates of acetylene reduction are linear over time. The short periods of time, e.g., 1-24 hours, and the sometimes destructive nature of the technique, i.e., the excavation of below-ground plant parts to expose them to the assay, limit extrapolation to intact systems over an entire growing season, although this assay may also be done on nondisturbed systems.

Acetylene reduction techniques have been applied to various field and laboratory conditions (38,60). Various reaction chambers are utilized, such as plastic bags (59), soft-drink cans (13), serum-vials (20) and soil cores (65). Problems with small plant samples, initial periods of inactivity (lag) and non-linear reduction rates, attributable in part to anaerobic nitrogen fixation, limit further extrapolation to field conditions (39). Periods of preincubation may alter the observed rates of nitrogen fixation. For example, Tjepkema and Van Berkum (65) observed a 14-fold higher estimation of fixation in preincubated vs. non-preincubated samples.

Nitrogen-15 enrichment and dilution techniques probably provide the most reliable estimates of nitrogen fixation. The major limitations are the high costs involved in reagents and in complex analyses. Such studies also require stringent controls to measure background ^{15}N incorporation levels, soil N mineralization and denitrification although such measurements are difficult to accomplish. The only major assumption is non-discrimination of ^{15}N and ^{14}N by plants and bacteria.

Enrichment experiments require that $^{15}N_2$, in a known excess concentration or $^{14}N_2$, be introduced into a closed or limited access vessel containing a nitrogen fixing system (22). The relative amount of ^{15}N is directly attributable to fixation; however, the elaborate vessels needed, the high cost of $^{15}N_2$, which limits time of exposure and number of samples, and the inability to provide natural field conditions make extrapolations difficult. Enrichment experiments are useful to calculate C_2H_2 conversion ratios for acetylene reduction measurements.

Dilution experiments require the application of nitrogen fertilizer containing a known excess of ^{15}N, usually 1 to 5%. As the fertilizer is taken up by the plant, any dilution of the original concentration of ^{15}N may be attributable to the utilization of soil nitrogen and to fixed, incorporated ^{14}N from the air (31). After a series of calculations (26,36) to derive "A" values, an estimate of nitrogen fixation is obtained. These estimates usually correlate with total nitrogen measurements. Williams et al. (73), using clover, found that Kjeldahl methods consistently underestimated ^{15}N dilution estimates by 40%. Dilution experiments are done on a much larger scale than enrichment experiments. Their advantages include a lower reagent cost, simple pot containers (dilution experiments may also be done under field conditions) and a long duration of exposure (20,21).

Legume-Rhizobium Symbioses

Most legumes, when in contact with the proper species and strains of Rhizobium bacteria, form root nodules. Nodules enclose the bacteroids, specialized forms of nondividing bacteria, and provide an environment for the reciprocal exchange of bacterial-fixed nitrogen

and plant-fixed carbon sources. One of the most important components of the nodule is leghemoglobin, a plant product similar to human hemoglobin, that binds O_2 and reduces its concentration to low levels, protecting rhizobia's oxygen sensitive nitrogenase, yet supplying adequate quantities of O_2 to maintain metabolic activities (15,23).

Well nodulated legumes fix agronomically important amounts of nitrogen and are therefore extremely important in world agriculture. Amounts of nitrogen fixed on a hectare basis range from 150 kg for soybean (36) to 295 kg for clover and 59 kg for vetch (73). Nodulation and nitrogen fixation are affected by many environmental and cultural conditions. In alfalfa and trefoil, Barta (6) observed approximately a 50% decrease in nitrogen fixation when the plants were grown at 30 C vs. 16 C. High levels of applied nitrogen and the location of application within the root zone may severely affect nodulation and subsequent nitrogen fixation. Molybdenum, a component of a nitrogenase co-factor, is also important and may easily become limiting in the field (52). Soil pH, pO_2, organic matter content and buffering capacity also affect symbiotic nitrogen fixation (23,69).

Studies designed to assess plant breeding potential for nitrogen fixation have been initiated with some major legumes. Wacek and Brill (69) screened 45 soybean cultivars in 6 maturity groups for nitrogen fixation. A broad range of relative acetylene reduction values were obtained, representing a 20-fold difference between the cultivars. Westermann and Kolar (72) also found a broad range of acetylene reduction values in 18 field-grown common bean cultivars belonging to several plant growth types and maturity groups. In 20 selfed and hybrid progenies of alfalfa, significant variation in nodule number

and acetylene reduction and a significant positive correlation between the two parameters have been observed (58). In a complete diallel cross between 6 progenies selected for high and low acetylene reduction rates, high X high crosses produced progenies with greater than twice the acetylene reduction rates than low X low crosses. High X low crosses produced progenies with intermediate rates. In another study (28), individual plants within the alfalfa cultivar 'Mesilla' were evaluated for nodulation, %N, total N and nitrogen fixation and positive correlations between these traits were observed. Polycross progeny from 15 plants selected for high trait levels showed a mean level of acetylene reduction 82% over the mean level for the entire population.

Grass-Bacterial Associations

Because of their tremendous potential, associative nitrogen fixing systems, involving various grasses and diazotrophs, have received a great deal of recent attention. Most associative studies have involved grass rhozosphere inoculation with previously isolated diazotrophs and the subsequent measurement of yield and nitrogen fixation by total N, acetylene reduction and ^{15}N methodology. Earlier studies have involved bahiagrass-*Azotobacter paspali* associations and a number of forage and grain crops in association with *Azospirillum brasilense* and *A. lipoferum* (formerly *Spirillum lipoferum*). Growth stimulation by these bacteria is thought by some to be due, at least in part, to bacterial plant growth regulator synthesis (5,17,63); however, many nitrogen fixation studies *per se* have been and continue to be done. In Brazil, Von Bulow and Dobereiner (68) screened corn genotypes in association with *Azospirillum* and indicated that sufficient variability existed in nitrogenase activity to warrant plant breeding. Nitrogenase

activities equivalent to the fixation of 0 to 734 gN/ha/day in Azospirillum inoculated corn have also been shown under greenhouse conditions in Oregon, while field activities were much lower (4). Schank et al. (55) also found nitrogenase activity differences in breeding lines of 30 tropical forage grasses in Brazil. In Florida, yield increases in some genotypes of Azospirillum inoculated pearl millet (12,13,59), guineagrass (59), bahiagrass (7) and bermudagrass (3) have been documented. Using fluorescent antibody labeling and conventional microscopy, bacteria in such associations have been observed in the root cortex and mucigel layer (56). Using electron microscopy, Azospirillum cells have been observed intercellularly in field-grown roots of pearl millet (46) and adsorbed to roots and root hairs and embedded in the mucigel layer of axenically grown pearl millet and guineagrass (67). Also using electron microscopy, the diazotroph Erwinia herbicola has been observed embedded in the root cell walls of switchgrass (43). In the rhizospheres of certain chromosome substitution lines of wheat, diazotrophs have been isolated that were not found associated with the chromosome donor or non-substituted lines (48). Other reports of associative nitrogen fixing systems include stargrass (42), rice (39), switchgrass (64) and Oryzopsis, Agropyron, Stipa and Aristida spp. in xeric habitats (74).

Many factors influence associative nitrogen fixing systems, much as those involved in legume symbioses. Field-applied nitrogen in amounts greater than 22 to 40 kg/ha significantly reduce responses to diazotroph inoculation (34,59). In vitro studies with rice (44) also indicate that nitrogen fixation is significantly inhibited by ammonium or nitrate in concentrations exceeding 50 ppm. This inhibition is

markedly less in water saturated vs. aerobic media. Associative systems may be temperature and light sensitive and may exhibit diurnal variations (2), but such data are highly variable and do not solidly support a positive conclusion. In addition to plant genetics, it is also thought that carbon metabolism pathways are important. In tropical associations, C_4 grasses are hypothesized by Day et al. (25) to have a competitive nitrogen fixation advantage over C_3 species, but no strong data to support such a hypothesis exist.

Other approaches to constitute new nitrogen fixing systems include genetic engineering. Using a plasmid involved in tumor induction by the crown gall bacterium, attempts to introduce nitrogen fixation genes into higher plants are being undertaken (57). Direct attempts to introduce nitrogen-fixing blue-green bacteria into corn and tobacco protoplasts have been made (19); however, plant regeneration with the incorporated bacteria is not yet possible. New, more precise approaches to reconstitute natural associative systems are also currently underway. Gilmour et al. (34), using diazotrophs isolated from native grass rhizospheres, have devised axenic and natural systems to test the patterns of root-bacterial associations. Plant-bacterial combinations that show a close association have been placed in the greenhouse and the field, and significant yield and nitrogenase activity increases have been observed.

Nitrogen Uptake Efficiency

Nitrogen uptake efficiency, the ability of a plant to recover applied, soil and fixed nitrogen, is a very important agronomic and plant breeding consideration. Reported efficiencies generally vary with species and genotype and range from 10 to 70%. In addition to species

and genotype differences (53), it has been documented that C_4 grasses have higher nitrogen efficiencies than C_3 grasses (18). An evolutionary advantage of C_4 over C_3 grasses has been postulated, based on relative recovery rates of various species. It is assumed in this hypothesis that C_4 photosynthesis evolved in tropical regions with soils low in nitrogen, and that nitrogen use efficiencies and this photosynthetic pathway are therefore linked. High nitrogen efficiencies in tropical regions with sandy soils and high rainfall, where typically less than 50% of applied nitrogen is actually recovered, are important plant traits (8). Also, in temperate regions with heavier soils that bind and immobilize applied nitrogen, high nitrogen uptake efficiencies are valuable (37,41). High nitrogen efficiencies are not always beneficial, however, and may result in toxic compound conditions under some conditions (35).

Time, rate of application and the nitrogen source used oftentimes influence efficient fertilizer use. Blue (9,10), using bahiagrass, observed low (40-50%) recoveries during the first four years of pasture establishment with recoveries of 60-70% after the fifth year. High recoveries were generally associated with higher rates of application, when applied during periods most favorable for plant growth. Efficiencies related to nitrogen source were noted, with the uptake of ureaform nitrogen markedly less than that of urea, calcium nitrate, ammonium nitrate and ammonium sulfate. Over a 25 year period with these experiments, soil nitrogen was increased by 800 kg/ha, indicating that nitrogen efficiency is related to nitrogen recycling and soil improvement (11).

Switchgrass

Switchgrass, Panicum virgatum L., is a member of the Virgata section of the Panicum subgenus Eupanicum. It is a large, bunch-type grass found in prairies, open ground, open woods and brackish marshes from Nova Scotia to Central America and west to North Dakota, Wyoming, Nevada and Arizona (40). Grown extensively in the midwestern United States for many years, released synthetic cultivars include 'Caddo,' 'Pathfinder,' 'Nebraska 28' and 'Blackwell' (1). Switchgrass pastures perform well in comparison to other native, adapted grasses and provide high yields of good quality forage with good stand persistence (71).

Switchgrass strains respond differently to soil types and cultural practices and respond well to nitrogen fertilization (48). In Nebraska, switchgrass yields were increased by approximately 75% with the application of 35 kgN/ha/year over a two year period (70). Percent N in switchgrass forage varies with strain, location of growth and nitrogen fertilization. Newell (50) observed 0.95%N in switchgrass forage compared to 0.85%N in bluestem forage from the same study; and McMurphy et al. (47) found switchgrass to be intermediate in %N when compared to bluestem, indiangrass and lovegrass. Heritabilities and expected gains from selection are usually high for collected genotypes of switchgrass with regard to yield, quality, disease resistance and desirable morphological traits, making breeding and selection profitable in many instances (29,49,51).

MATERIALS AND METHODS

Plant Materials and Field Cultural Conditions

Six accessions of switchgrass were used as the base population for this study (Table 1). Large, field-grown plants, one of each genotype, were split into 12 uniform clones and established on 0.6 m centers in a completely randomized polycross field block in June, 1977. In October 1977, mature spikelets were harvested and equal amounts of seed from each maternal plant were mixed and pooled into 6 lots. These half-sib progenies were established in flats of non-amended field soil in the greenhouse. Randomly chosen seedlings were placed individually in cell-pack flats containing non-amended field soil, lightly fertilized with the equivalent of 400 kg/ha 6-6-6 organic fertilizer and allowed to become well established.

The ramets were planted in the field at Gainesville and hague, Florida, in May 1978. The Gainesville location is well-drained Gainesville sand, loamy, hyperthermic Typic Quartzipsamments, pH 4.5-6.0, with an analyzed soil N content of 0.046%. The Hague location is Sparr sand, loamy, siliceous, hyperthermic Grossarenic Paleudalts, pH 4.5-6.5, with an analyzed soil N content of 0.056%, and is susceptable to partial flooding after a heavy rain. Percent N in each soil type did not change over the duration of the experiment. These locations were chosen because of their diversity in soil type and moisture relationships to adequately test the genotypes. Prior to planting, all field locations were sprayed with 5 l/ha of glyphosate herbicide, plowed 10 days later, fertilized with the equivalent of 1,000 kg/ha of 0-10-20 fertilizer with fritted

Table 1. Plant Materials Center accession numbers of the Switchgrass (*Panicum virgatum* L.) parental clone genotypes and their locations of collection.

Accession number*	Location of collection
F-687	Stuart, FL
F-1666	West Palm Beach, FL
F-1668	Ft. Pierce, FL
F-1716	Arcadia, FL
F-4685	George West, TX
F-3115	Miami, FL

*All accessions were obtained courtesy of R. D. Roush, Manager, U.S. Soil Conservation Service Plant Materials Center, Brooksville, FL.

trace elements (FTE 503; 5 B: 5 Cu: 29 Fe: 12 Mn: 0.3 Mo: 11 Zn, in g/100kg) and lightly disced.

The field trials consisted of 3 fertilizer N levels applied to 6 polycross half-sib lines of switchgrass, and a bahiagrass check species (Paspalum notatum Flugge cv 'Pensacola'). The total number of switchgrass plants evaluated was 1,260. Fertilizer N levels were the equivalent of 10, 50 and 90 kg/ha elemental N applied by hand as NH_4NO_3. Bahiagrass was obtained as 7.5 cm plugs from a well-established sod. Plots were 4 m long and 1 m wide and consisted of 7 ramets per plot on 0.6 m centers with 5 completely randomized block replications at the 2 locations over a 2 year period. Plots were separated by 2 m and during 1978, a border row of a sorghum-sudangrass hybrid was included between each plot. Border rows were omitted in 1979. Plots were irrigated as needed during early establishment in 1978. Weeds were controlled by mowing and hand-pulling. Forage was harvested in October of both years with a flail-type plot harvester for yield and nitrogen analyses. October was chosen as a harvest date in order to obtain mature seeds from the plants for further studies.

Field Selection and Analysis of Acetylene Reduction Activity (AR)

During the 1978 growing season, each switchgrass plant was rated several times on the basis of vigor, decumbance, lateness of flowering and color. Vigor was scored on 5 levels (1,3,5,7,9). Decumbance, a desirable trait for a bunch-type forage grass, was scored on 2 levels (0 for completely upright types and 1 for any degree of spreading). Lateness of flowering, also a desirable trait, was scored on 2 levels

(0 for plants flowering before August 15 and 1 for those that flowered afterwards). Color, thought to be a neutral trait, was scored as either blueish or completely green. Broad-sense heritabilities for the numerically scored traits were calculated. The most highly rated plant in each plot, representing the best 1 out of 7 or the top 14%, was used to generate further plant material, and the most highly rated plants in plots of 2 replications at each location were sampled for AR in 1978. In 1979, the most highly rated plants in plots of all 5 replications were sampled.

The AR sampling procedure involved taking soil-root cores in tubes, consisting of a 7.5 cm diameter piece of steel electrical conduit, 36 cm long. One end of the core tube had either a welded steel top or a No. 13 rubber stopper with a sampling tube for the insertion of a rubber septum. The opposite end of the tube was sharpened. At sampling time, the cores were taken by gently pressing the tubes into the root zone of each plant to a depth of 18 cm, and carefully removing and sealing with a Jim-Cap secured with a steel hose clamp. Each core was then freely flushed with argon for 1 min through the top sampling tube and sealed with the septum. Acetylene was added through the septum to an approximate concentration of 10% (v/v). Cores were incubated at 30 C in a growth chamber. Internal atmospheres were monitored for ethylene evolution by gas chromatography at various incubation times. A lag time of approximately 5 hours was commonly observed, and the rates of acetylene reduction were linear after the lag and up to 24 hours. A core consisting of bare soil was included after every 21 plant samples, and always exhibited zero or near zero rates of acetylene reduction. Extrapolations of N fixed were all based on 24 hour readings and included

the assumptions of a theoretical ratio of 3.0 moles ethylene per mole N_2, 18 hours of activity per day and 22 x 10^6 cores per ha.

Greenhouse Evaluations

Four populations of switchgrass selected under various selection intensities (as described in Table 2) and bahiagrass plugs were established with 5 replications in 15 cm plastic pots, and randomly placed in the greenhouse in March, 1979. Populations 1, 2 and 3 were derived as previously discussed. Population 4 consisted of the best 1 out of 100 seedlings generated from field-grown seed from plants of population 3. The plants were outcrossed, but not in a true polycross layout, and some inbreeding is therefore expected in population 4. A polycross block was not established in order to obtain a maximum number of populations without extending the work an additional season.

All pots contained uniform amounts of screened, non-amended field soil, later fertilized with the equivalent of 1,000 kg/ha of 0-10-20 with FTE 503 and 10 kg/ha of N. Soil N content was analyzed before fertilization at 0.050% and did not change over the duration of the experiment. Nitrogen was applied in aqueous solution as $(NH_4)_2SO_4$ with a 3.00 atom % excess of ^{15}N. Homogenate of excavated field-grown roots from plots exhibiting the highest levels of AR was added to the pots at the time of fertilization to insure the presence of diazotrophic bacteria. During the course of the experiment, any leachate was returned to the pots. Verdure was harvested in July, 1979, N fertilizer and fresh root homogenate reapplied, and the regrowth harvested in October. The material from each harvest was dried and weighed for yield, pooled over the 5 replications, and analyzed for total N and ^{15}N content.

Table 2. Description of the populations used in the greenhouse experiments and their selection intensities.

Population	Description	Selection Intensity
1	Original parental clones (see Table 1).	--
2	Randomly selected poly-cross progeny from population 1.	0%
3	Field selected individuals from population 2.	86%
4	Greenhouse selected out-crossed progeny from population 3.	99%

Total N and ^{15}N Determinations

For total nitrogen, forage samples from field plots and the pooled samples from the greenhouse pots were dried at 60 C and ground through a Wiley mill to pass a 1 mm mesh stainless steel screen. Sub-samples of the mixed, ground material, weighing 0.1 g, were placed in test tubes containing 2-3 boiling chips and 3.2 g of a K_2SO_4 : $CuSO_4$ (9:1 w/w) catalyst. After the addition of 10 ml conc. H_2SO_4 and 2 ml 30% H_2O_2, the samples were digested on an aluminum block at 360C, to give a solution boiling temperature of approximately 342C, for 3 hours. The digestates were diluted to 75 ml with deionized water and analyzed by colorimeter autoanalyzer (32). This N content data were used to calculate gross fertilizer use efficiency by the equation:

$$[(\%N)\ (\text{dry matter yield})\ 100^{-1}\ \ (\text{rate of N applied})^{-1}].$$

Such N fertilizer use efficiencies do not discriminate between soil, applied and fixed N and are therefore only an overall estimate of this parameter.

Total nitrogen in field and greenhouse soils was determined by digesting 1 g of screened soil in the manner above. After the addition of 15 ml of 10 M NaOH, 30 ml of the digestate was steam distilled into 5 ml 0.1 M H_3BO_3 and 2 drops of mixed indicator (2 parts 0.2% methyl red and 1 part 0.2% methylene blue in ethanol) and titrated to end-point with 0.0065 N H_2SO_4 (14).

Nitrogen-15 determinations were made on 0.2 g of dried, ground plant material. Samples were placed in test tubes containing 1-3 boiling chips and 1.5 g K_2SO_4. After the addition of 1.5 ml mercuric

sulfate solution ($12H_2SO_4$: $88H_2O$: 10HgO v/v/w) and 3 ml of conc. H_2SO_4, the samples were digested on aluminum block at 360C for 3 hours and then diluted with 25 ml deionized H_2O. The digestates were neutralized with 15 ml 10 M NaOH and steam distilled into 10 ml of 0.01 N H_2SO_4. The distillates were condensed to ca. 0.5 ml and dried on 1 x 11 cm filter paper strips. Ammonium on the strips was converted to N_2 gas with 1.5 ml of alkaline hypobromite (8Br : 40 13 N NaOH : $30H_2O$ v/v/v) and analyzed for ^{15}N atom% excess with a Consolidated-Nier isotope ratio mass spectrometer (20,21,22).

Nitrogen-15 atom % excess and ^{15}N "A" values were used in the analyses (31,36,73). "A" values were calculated with the equation:

$$[(\text{total N} - \text{fertilizer N})\ (\text{fertilizer N} \div \text{rate of applied N})^{-1}] .$$

Estimated N fixed was calculated by the equation:

$$[(\text{"A" for the fixing plant} - \text{"A" for the control})\ (\text{fertilizer N})\ (\text{rate of applied N})^{-1}] .$$

Since a suitable control is not available in studies involving associative nitrogen fixation, the control values used in the above equation were assumed to be zero. The implications of this assumption will be discussed. Additional calculations of N fertilizer use efficiency were made using the ^{15}N data with the equation:

$$[(\text{total plant N})\ (\text{atom \% } ^{15}N)\ (\text{amount of } ^{15}N \text{ applied})^{-1}] .$$

Such N fertilizer use efficiencies discriminate between applied and other forms of N and are a more accurate assessment of actual fertilizer uptake.

RESULTS AND DISCUSSION

Field Evaluations

A summary of the analysis of variance for yield, %N, fertilizer use efficiency (calculated by total N) and estimates of nitrogen fixation by total N difference and acetylene reduction, combined over both locations and both years, is presented in Table 3. Main effects, i.e. location, fertilizer, line and year were significant for yield, fertilizer use efficiency and the estimate of nitrogen fixation by total N difference. Location, line and year significantly influenced %N but nitrogen fertilizer rate did not. Estimated nitrogen fixation by acetylene reduction showed no influence by main effects and only the line X year interaction was significant. Two-factor interactions showed mixed effects for the other parameters. The location X line (environment X genotype) interaction is an extremely important one and was significant for yield, %N and fertilizer use efficiency, indicating the need for multiple test sites for switchgrass in further evaluations. Both estimates of nitrogen fixation failed to show significant location X line interactions, however, and these parameters may be assumed to be relatively constant. Although both locations responded the same in both years, yield and %N were not consistent over locations and fertilizers, indicating a need for specialized fertilizer requirements due to different soil types. Significance in the year X fertilizer and year X line interactions indicate that plant

Table 3. Summary of the analysis of variance for yield, %N, N fertilizer use efficiency (FEFF) and estimates of nitrogen fixation by total N methods (NF TN) and acetylene reduction (NF AR) of main effects and two-factor interactions in the field experiments.

SOURCE	d.f.	YIELD	%N	FEFF	NF TN	NF AR
LOCATION	1	**	**	**	*	NS
FERTILIZER	2	**	NS	**	**	NS
LINE	6	**	**	**	**	NS
YEAR	1	**	**	**	**	NS
LOCATION X FERTILIZER	2	*	NS	**	NS	NS
LOCATION X LINE	6	**	**	**	NS	NS
FERTILIZER X LINE	12	**	*	**	*	NS
LOCATION X YEAR	1	NS	NS	*	*	NS
FERTILIZER X YEAR	2	**	NS	**	NS	NS
LINE X YEAR	6	**	**	**	*	*

*,** are significant at the 5% and 1% levels of probability, respectively.
NS, not significant.

establishment over the two years is important in yield and N fertilizer response. Percent N and nitrogen fixation are also significantly influenced by plant establishment.

Half-sib switchgrass lines and bahiagrass varied significantly with regards to yield, %N, N fertilizer use efficiency and nitrogen fixation estimated by total N difference (Table 4). All switchgrass yielded significantly more dry matter than bahiagrass, but the latter was higher in %N, indicating the ability of switchgrass to dilute nitrogen. Nitrogen dilution, as defined by Terman and Allen (61) refers to a relatively high concentration of N in young plants that becomes lower with increasing dry matter production and age. This type of nitrogen dilution should not be confused with ^{15}N dilution estimates of nitrogen fixation. Nitrogen dilution is particularly noticeable in pot experiments having a finite volume of soil for root development and occurs more rapidly under pot vs. field conditions. Dilution has been observed in corn (60,61) and Italian ryegrass (24). The present data show nitrogen dilution with reference to a species difference between switchgrass and bahiagrass in the field, in addition to limited half-sib line dilution differences within switchgrass. This ability, coupled with the high fertilizer use efficiency of switchgrass (Table 4), may be why this species does so well on poor soils with little or no nitrogen input. Switchgrass was also higher than bahiagrass in nitrogen fixation estimated by total N difference and showed a trend towards higher acetylene reduction.

Applied fertilizer nitrogen had a significant positive influence on crop yield, a negative influence on fertilizer use efficiency and no influence on %N (Table 5). Nitrogen fixation estimates were also negatively affected by fertilizer nitrogen in agreement with other observations with grasses (59) and legumes (52). This effect was

Table 4. Mean Yield, %N, N fertilizer use efficiency (FEFF) and estimates of nitrogen fixation by total N methods (NF TN) and acetylene reduction (NF AR) by lines in the field experiments.

LINE	YIELD kg/ha	%N	FEFF %	NF TN kg/ha	NF AR kg/ha
F-3115	7954a*	0.84c	255a	28.0ab	3.6a
F-4685	7381b	0.88bc	245ab	26.9ab	3.7a
F-1116	6543c	0.89b	223bc	34.9a	23.2a
F-1666	5869d	0.88bc	198c	16.9b	4.4a
F-1668	5776d	0.92ab	224bc	19.1ab	3.9a
F-687	5731d	0.92ab	209c	18.6ab	3.9a
Bahiagrass	945e	0.93a	35d	0.9c	2.7a

*Means followed by different letters are significantly different at the 5% level by Duncan's Multiple Range Test.

Table 5. Mean Yield, %N, N fertilizer use efficiency (FEFF) and estimates of nitrogen fixation by total N methods (NF TN) and acetylene reduction (NF AR) by fertilizer treatment in the field experiments.

FERTILIZER kgN/ha	YIELD kg/ha	%N	FEFF %	NF TN kg/ha	NF AR kg/ha
90	6434a*	0.88a	61c	5.2c	3.6a
50	5828b	0.91a	104b	17.1b	3.7a
10	4965c	0.90a	429a	39.9a	12.3a

*Means followed by different letters are significantly different at the 5% level by Duncan's Multiple Range Test.

significant for each increment of applied N when estimated by total N difference but showed only a trend in the acetylene reduction measurements.

All measured parameters, excluding the nitrogen fixation estimate by acetylene reduction, showed significant effects due to year and location. The significantly higher values obtained in 1979 (Table 6) probably relate most importantly to plant establishment. Plants grown at the Gainesville location were higher in yield, fertilizer use efficiency and nitrogen fixation by total N difference (Table 7). Since the Hague location represents a poorly drained site, the sometimes waterlogged soil seemed to account for decreases in yield, and inhibited root development that would decrease plant uptake of N and other nutrients, including water. Percent N, however, was significantly higher at Hague, possibly due to a higher overall soil N content.

In the correlation analysis, yield and percent N showed a significant negative correlation while yield and fertilizer use efficiency and nitrogen fixation estimated by total N difference were positively correlated, as was the latter with %N (Table 8). Positive correlation ($p > 0.001$) exists between the two nitrogen fixation estimates. Although the relative magnitude of the values differs greatly (Tables 5, 6, 7, 8), their correlation indicates that both are applicable to these systems and may be used concurrently or alone.

Broad-sense heritabilities (Table 9) were low for most of the parameters measured in the field. Only decumbance and lateness of flowering exhibited relatively high heritabilities that were stable over locations. The surprisingly low values for yield at Hague and the combined locations illustrates the effects of inhibited plant growth

Table 6. Mean Yield, %N, N fertilizer use efficiency (FEFF) and estimates of nitrogen fixation by total N methods (NF TN) and acetylene reduction (NF AR) by year in the field experiments.

YEAR	YIELD kg/ha	%N	FEFF %	NF TN kg/ha	NF AR kg/ha
1979	8297a*	0.92a	293a	32.5a	4.0a
1978	3187b	0.87b	103b	9.1b	12.8a

*Means followed by different letters are significantly different at the 5% level by Duncan's Multiple Range Test.

Table 7. Mean Yield, %N, N fertilizer use efficiency (FEFF) and estimates of nitrogen fixation by total N methods (NF TN) and acetylene reduction (NF AR) by location in the field experiments.

LOCATION	YIELD kg/ha	%N	FEFF %	NF TN kg/ha	NF AR kg/ha
GAINESVILLE	7510a*	0.85b	243a	25.3a	2.4a
HAGUE	3976b	0.95a	153b	16.3b	10.6a

*Means followed by different letters are significantly different at the 5% level by Duncan's Multiple Range Test.

Table 8. Pearson correlation coefficients (and their probabilities) for yield, %N, N fertilizer use efficiency (FEFF) and estimates of nitrogen fixation by total N methods (NF TN) and acetylene reduction (NF AR) in the field experiments.

	%N	FEFF	NF TN	NF AR
YIELD	-0.176 (>0.001)	0.409 (>0.001)	0.335 (>0.001)	-0.069 (0.267)
%N		0.068 (0.163)	0.112 (0.022)	0.041 (0.419)
FEFF			0.455 (>0.001)	-0.047 (0.419)
NFKJ				0.835 (>0.001)

Table 9. Broad-sense heritabilities, in percent, for yield, vigor, decumbance, lateness of flowering, %N, N fertilizer use efficiency by total N difference (FEFF TN) and estimates of nitrogen fixation by total N difference (NF TN) and by acetylene reduction (NF AR) in the field experiments at Gainesville and Hague and the combined locations.

	GAINESVILLE	HAGUE	COMBINED LOCATIONS
YIELD	22.2	8.6	6.8
VIGOR	44.6	22.7	3.9
DECUMBANCE	70.1	62.0	68.2
LATENESS OF FLOWERING	56.8	90.7	65.3
%N	13.2	5.3	5.9
FEFF TN	0.3	0.2	2.9
NF TN	0.8	4.3	5.1
NF AR	0.2	8.5	6.1

within a location and a very large location variance on heritability estimates. It is evident from these data that different switchgrass lines, in terms of yield, are suited to different locations, and that breeding progress would be slower under Hague conditions. This same sort of situation exists for %N. Heritabilities for fertilizer use efficiency and the estimates of nitrogen fixation are very low, as may be expected, and indicate that any breeding progress for these traits would be slow.

Greenhouse Evaluations

Mean yields, %N, fertilizer use efficiencies and estimates of nitrogen fixation by ^{15}N and Kjeldahl N methods for the switchgrass lines and bahiagrass in the first, second and combined harvests are presented in Tables 10, 11 and 12, respectively. The plants were at a pre-flowering stage of maturity when harvested. Although ample time for flowering was allowed (120 days for harvest 1 and 92 days for harvest 2) a delay was brought about by the greenhouse environmental conditions and time of year. Significant variations in yield and consistent yield ranking by lines were noted throughout the experiment. As in the field results, bahiagrass yielded less than switchgrass, but not as markedly. In addition, switchgrass line rankings were different between the greenhouse and the field. Percent N in switchgrass was significantly lower than that of bahiagrass, indicating that switchgrass is able to dilute nitrogen to a great extent. This, again, is one reason why switchgrass grows well under a low nitrogen input and makes the species an excellent biomass producer on poor soils. The extremely low %N values observed for pot-grown plants is in agreement with other published reports (24,61,62) and agrees with the species differences shown in the field.

Table 10. Mean Yield, %N, N fertilizer use efficiencies by ^{15}N uptake (FEFF ^{15}N), N fertilizer use efficiencies by total N difference (FEFF TN) and estimates of nitrogen fixation by ^{15}N dilution (NF ^{15}N) and total N difference (NF TN) by lines in the first greenhouse harvest.

LINE	YIELD g/pot	%N	FEFF ^{15}N %	FEFF TN %	NF ^{15}N gN/ha/day	NF TN gN/ha/day
F-687	8.80a*	0.36b	13.96a	176.75a	72.39a	64.33a
F-4685	7.78ab	0.35b	13.39a	146.25ab	73.08a	61.93a
F-1666	6.83abc	0.37b	13.34a	136.00ab	73.40a	29.65a
F-1116	6.66abc	0.33b	13.45a	119.75ab	71.77a	32.85a
F-1668	6.09bc	0.38b	12.76a	125.00ab	73.29a	22.21a
F-3115	6.00bc	0.34b	12.21a	110.50b	72.33a	13.05a
Bahiagrass	5.79c	0.43a	17.54a	136.80ab	72.28a	31.14a

*Means followed by different letters are significantly different at the 5% level by Duncan's Multiple Range Test.

Table 11. Mean yield, %N, N fertilizer use efficiencies by ^{15}N uptake (FEFF ^{15}N), N fertilizer use efficiencies by total N difference (FEFF TN) and estimates of nitrogen fixation by ^{15}N dilution (NF ^{15}N) and total N difference (NF TN) by lines in the second greenhouse harvest.

LINE	YIELD g/pot	%N	FEFF ^{15}N %	FEFF TN %	NF ^{15}N gN/ha/day	NF TN gN/ha/day
F-687	3.59a*	0.48b	19.89a	74.75a	88.66a	5.31a
F-1666	3.26a	0.46b	20.47a	81.75a	87.29a	0.00a
F-4685	3.07a	0.44b	19.90a	77.75a	87.63a	0.58a
F-3115	2.94ab	0.47b	20.66a	74.25a	90.01a	0.00a
F-1116	2.80ab	0.48b	19.48a	81.75a	90.00a	0.00a
F-1668	2.72ab	0.52b	20.98a	73.25a	86.35a	23.91a
Bahiagrass	2.37b	0.62a	20.41a	80.74a	73.14b	0.00a

*Means followed by different letters are significantly different at the 5% level by Duncan's Multiple Range Test.

Table 12. Mean yield, %N, N fertilizer use efficiencies by ^{15}N uptake (FEFF ^{15}N), N fertilizer use efficiencies by total N difference (FEFF TN) and estimates of nitrogen fixation by ^{15}N dilution (NF ^{15}N) and total N difference (NF TN) by lines in the combined greenhouse harvests.

LINE	YIELD g/pot	%N	FEFF ^{15}N %	FEFF TN %	NF ^{15}N gN/ha/day	FN TN gN/ha/day
F-687	6.19a*	0.42b	16.92a	125.75a	80.53a	34.82a
F-4685	5.42b	0.39b	16.64a	112.00a	80.35a	31.25a
F-1666	5.04bc	0.41b	16.95a	108.88a	80.34a	14.83a
F-1116	4.73bc	0.40b	16.46a	100.75a	80.89a	16.42a
F-3115	4.47cd	0.40b	16.44a	92.38a	81.17a	6.53a
F-1668	4.40cd	0.45b	16.87a	99.13a	79.82a	23.06a
Bahiagrass	4.08d	0.52a	18.98a	108.77a	72.71b	15.57a

*Means followed by different letters are significantly different at the 5% level by Duncan's Multiple Range Test.

Fertilizer N use efficiencies estimated by ^{15}N uptake were low throughout the experiment and no line differences were observed. This measurement includes only fertilizer N uptake, and is not confounded with fixed or soil N. Nitrogen efficiencies estimated by total N difference were higher than ^{15}N uptake values, but are confounded with applied, fixed and soil N, which are not separable by the total N method. It is assumed that the N uptake values are most accurate; however, it seems unrealistic that less than 25% of the applied N was actually recovered in the verdure, especially since leaching was avoided. It may be possible that applied N was incorporated into stable, non-available soil N forms and a turnover of non-labeled soil N was released for plant uptake. Further studies would be needed to support this hypothesis.

Estimates of nitrogen fixation were also low; however, in the second and combined harvests, nitrogen fixation (by ^{15}N dilution) was significantly lower in bahiagrass than in switchgrass, indicating an important advantage of switchgrass over bahiagrass under nitrogen limited conditions. This result is in agreement with field estimates of nitrogen fixation in the two species. No overall differences existed between switchgrass lines for %N, fertilizer use efficiency or nitrogen fixation. This may be indicative of a species trait or a starting population of plants with low genetic variance.

From a plant breeding standpoint, some extremely important values are obtained when switchgrass lines are pooled over the four populations. In the first harvest (Table 13), significant differences due to population were found for yield, fertilizer use efficiency and nitrogen fixation. Since selection procedures were visual, and included yield as one criterion, increases in yield are expected, and populations 3 and

Table 13. Mean yield, %N, N fertilizer use efficiencies by ^{15}N uptake (FEFF ^{15}N), N fertilizer use efficiencies by total N difference (FEFF TN) and estimates of nitrogen fixation by ^{15}N dilution (NF ^{15}N) and total N difference (NF TN) by populations in the first greenhouse harvest.

POPULATION	YIELD g/pot	%N	FEFF ^{15}N %	FEFF TN %	NF ^{15}N gN/ha/day	NF TN gN/ha/day
1	9.23a*	0.36a	15.76a	184.67a	70.34b	70.82a
3	7.27b	0.34a	10.29b	134.17b	74.54a	42.74ab
4	6.81b	0.34a	13.35ab	126.67b	73.60ab	32.89ab
2	4.80c	0.37a	13.39ab	97.33b	72.36ab	2.90b

*Means followed by different letters are significantly different at the 5% level by Duncan's Multiple Range Test.

4 were significantly higher than the unselected population 2 in the first and combined harvests (Table 14), although the selected populations did not exceed population 1. This may be due to a highly selected population 1, which is reasonable to assume since a plant collector would intuitively select the visually best plants within a location.

In addition to yield, fertilizer efficiency by ^{15}N uptake in population 1 was higher than population 3, although populations 2, 3 and 4 did not differ indicating no selection improvement for this trait. The most exciting observations are in the estimates of nitrogen fixation by ^{15}N dilution. In the first harvest, population 3 was significantly higher than population 1 for nitrogen fixation, and in the combined harvests, population 4 significantly exceeded population 1. In the second harvest (Table 15), nitrogen fixation was also highest in population 4 and significantly exceeded population 3, but was not different from populations 1 or 2. The results suggest that selection for nitrogen fixation may be possible but will require further refinement of the measurement techniques and understanding of the cultural conditions (e.g., plant establishment, growth environment, time of year) that affect genetic expression of associative nitrogen fixing abilities. For example, harvest time played an important role in all traits measured (Table 16). Yield and estimated nitrogen fixation by the total N methodwere significantly higher in harvest 1, due to better plant growth, whereas growth was restricted by the pots in harvest 2. While total N difference calculations did not agree, estimated nitrogen fixation by the more sensitive and reliable ^{15}N dilution method was higher in harvest 2 and illustrates the importance of time of year and plant establishment on this trait. Higher %N and fertilizer use efficiency by ^{15}N uptake

Table 14. Mean yield, %N, N fertilizer use efficiencies by ^{15}N uptake (FEFF ^{15}N), N fertilizer use efficiencies by total N difference (FEFF TN) and estimates of nitrogen fixation by ^{15}N dilution (NF ^{15}N) and total N difference (NF TN) by populations in the combined greenhouse harvests.

POPULATION	YIELD g/pot	%N	FEFF ^{15}N %	FEFF TN %	NF ^{15}N gN/ha/day	NF TN gN/ha/day
1	6.30a*	0.43a	18.38a	134.25a	79.38b	45.34a
3	5.21b	0.40a	15.87a	105.83b	79.61ab	21.37ab
4	4.82b	0.39a	15.93a	98.17b	82.40a	16.45b
2	3.84c	0.43a	16.67a	87.67b	80.67ab	1.45b

*Means followed by different letters are significantly different at the 5% level by Duncan's Multiple Range Test.

Table 15. Mean yield, %N, N fertilizer use efficiencies by ^{15}N uptake (FEFF ^{15}N), N fertilizer use efficiencies by total N difference (FEFF TN) and estimates of nitrogen fixation by ^{15}N dilution (NF ^{15}N) and total N difference (NF TN) by populations in the second greenhouse harvest.

POPULATION	YIELD g/pot	%N	FEFF ^{15}N %	FEFF TN %	NF ^{15}N gN/ha/day	NF TN gN/ha/day
1	3.38a*	0.48a	21.00a	88.83a	88.41ab	19.86a
3	3.15a	0.46a	21.46a	77.50a	84.69b	0.00a
2	2.88a	0.50a	19.45a	78.00a	88.98ab	0.00a
4	2.83a	0.44a	18.51a	69.67a	91.20a	0.00a

*Means followed by different letters are significantly different at the 5% level by Duncan's Multiple Range Test.

Table 16. Mean Yield, %N, N fertilizer use efficiencies by ^{15}N uptake (FEFF ^{15}N), N fertilizer use efficiencies by total N difference (FEFF TN) and estimates of nitrogen fixation by ^{15}N dilution (NF ^{15}N) and total N difference (NF TN) by harvest in the greenhouse experiment.

HARVEST	YIELD g/pot	%N	FEFF ^{15}N %	FEFF TN %	NF ^{15}N gN/ha/day	NF TN gN/ha/day
1	7.03a*	0.35b	13.20b	135.70a	72.71b	37.34a
2	3.06b	0.47a	20.22a	77.25b	88.32a	4.97b

*Means followed by different letters are significantly different at the 5% level by Duncan's Multiple Range Test.

in harvest 2 also indicates the effect of plant establishment on these parameters and includes the utilization of soil nitrogen during the first growth period.

Correlation analyses for the six parameters in harvests 1, 2 and the combined harvests are presented in Tables 17, 18 and 19, respectively. In the combined harvests, yield and %N were negatively correlated, indicating nitrogen dilution. Yield and fertilizer use efficiency by ^{15}N uptake, while positively correlated in harvest 2, showed an overall negative correlation, and fertilizer efficiency was positively related to %N in the combined harvests. Nitrogen fixation by ^{15}N dilution was, over both harvests, negatively correlated to yield and positively so to %N, indicating that these factors may be of value in initial selection and screening procedures in a breeding program. Yield may be sacrificed to support associative diazotrophs, as previously hypothesized by Brill (15). In contrast, nitrogen fixation estimated by the total N method was positively correlated to yield in the first and combined harvests indicating again the need for technique refinement. This and the non-agreement and overall negative correlation of total N and ^{15}N dilution estimates indicate the relative insensitivity of the Kjeldahl method. The importance of such measurements lies in an overall estimation of gross nitrogen relationships.

In the combined greenhouse harvests (Table 14), mean estimates of nitrogen fixation by the total N method were 75% lower than ^{15}N dilution estimates. This is in contrast to the 40% underestimates observed by Williams et al. (73) using legume systems. In "A value" calculations (see Materials and Methods section) involving legume systems, either a grass or non-nodulating legume is used as a control, and includes N

Table 17. Pearson correlation coefficients (and their probabilities) for Yield, %N, N fertilizer use efficiencies by ^{15}N uptake (FEFF ^{15}N), N fertilizer use efficiencies by total N difference (FEFF TN) and estimates of nitrogen fixation by ^{15}N dilution (NF ^{15}N) and total N difference (NF TN) in the first greenhouse harvest.

	%N	FEFF ^{15}N	FEFF TN	NF ^{15}N	NF TN
YIELD	-0.033 (0.879)	0.392 (0.058)	0.936 (>0.001)	-0.214 (0.316)	0.851 (>0.001)
%N		0.332 (0.113)	0.304 (0.149)	0.049 (0.820)	0.184 (0.390)
FEFF ^{15}N			0.478 (0.018)	-0.559 (0.005)	0.358 (0.086)
FEFF KJ				-0.169 (0.429)	0.884 (>0.001)
NF ^{15}N					-0.094 (0.663)

Table 18. Pearson correlation coefficients (and their probabilities) for Yield, %N, N fertilizer use efficiences by ^{15}N uptake (FEFF ^{15}N), N fertilizer use efficiencies by total N difference (FEFF TN) and estimates of nitrogen fixation by ^{15}N dilution (NF ^{15}N) and total N difference (NF TN) in the second greenhouse harvest.

	%N	FEFF ^{15}N	FEFF TN	NF ^{15}N	NF TN
YIELD	-0.332 (0.113)	0.467 (0.021)	0.004 (0.987)	-0.327 (0.119)	0.051 (0.811)
%N		0.040 (0.852)	0.214 (0.315)	-0.060 (0.781)	0.425 (0.039)
FEFF ^{15}N			0.089 (0.678)	-0.734 (>0.001)	-0.015 (0.947)
FEFF KJ				-0.165 (0.440)	-0.062 (0.773)
NF ^{15}N					0.123 (0.567)

Table 19. Pearson correlation coefficients (and their probabilities) for Yield, %N, N fertilizer use efficiencies by ^{15}N uptake (FEFF ^{15}N), N fertilizer use efficiencies by total N difference (FEFF TN) and estimates of nitrogen fixation by ^{15}N dilution (NF ^{15}N) and total N difference (NF TN) in the combined greenhouse harvests.

	%N	FEFF ^{15}N	FEFF TN	NF ^{15}N	NF TN
YIELD	-0.663 (>0.001)	-0.446 (0.002)	0.926 (>0.001)	-0.628 (>0.001)	0.762 (>0.001)
%N		0.676 (>0.001)	-0.426 (0.003)	0.546 (>0.001)	-0.218 (0.134)
FEFF ^{15}N			-0.306 (0.035)	0.249 (0.091)	-0.179 (0.225)
FEFF KJ				-0.529 (>0.001)	0.819 (>0.001)
NF ^{15}N					-0.529 (>0.001)

inputs by associative and free-living nitrogen fixation in addition to the contribution by soil N. In this study, no suitable control was available and the ^{15}N dilution estimates are, therefore, absolute ones. Originally, bahiagrass was to be used as a control in these calculations based on prior observations of low acetylene reduction in this species (7); however, some bahiagrass "A" values were higher than those of some switchgrasses. Also, the relative contribution of soil and fixed N in the ^{15}N nitrogen fixation estimates is not known.

It would also be desirable to have acetylene reduction data for the greenhouse experiment. Attempts to obtain such data were made in large plexiglass chambers designed to accomodate a 15 cm pot. Nearly 300 such measurmeents were made; however, the data were rendered invalid by a blue-green bacterial bloom during the first growth period and a general non-response in the second, possibly due to the root-bound nature of the pots. The latter observation was not, however, consistent with ^{15}N dilution data (Table 16) which indicate more nitrogen fixation in harvest 2 than in harvest 1, when the plants were better established. Since the blue-green bacterial crusts were scraped from the soil surface and discarded, their fixed nitrogen may have also been removed, a speculation consistent with these data.

CONCLUSIONS

Combined field and greenhouse studies with switchgrass breeding lines and Pensacola bahiagrass showed differences in yield, %N, fertilizer use efficiency and nitrogen fixation measured by three methods: total nitrogen difference, acetylene reduction and ^{15}N dilution. In the field, nitrogen fixation estimates by the total N method and acetylene reduction showed a significant positive correlation, but in the greenhouse, total N methods were negatively correlated to ^{15}N dilution estimates, indicating a great need for further refinement of these techniques. Mean estimates of the amount of nitrogen fixed by the total N method were 20.8 and 2.4 kgN/ha in the field and greenhouse, respectively. Acetylene reduction estimated a mean of 6.5 kgN/ha fixed in the field, while ^{15}N dilution in the greenhouse estimated 9.5 kgN/ha. The results verify that an agronomically significant amount of nitrogen is being fixed by indigenous diazotrophs in the rhizospheres of these forage grasses.

From a plant breeding standpoint, improvement of switchgrass for yield and fertilizer use efficiency seems possible. Improvement for associative nitrogen fixation also appears possible, but the results of a breeding and selection program will be dependent, in part, on the type of measurements made and the environmental and cultural conditions. Broad-sense heritabilities for these parameters were generally low, indicating that such breeding progress will be slow. Overall results

indicate the need for further collection of a broad-based germplasm switchgrass population.

Switchgrass yields well on sandy soils under low nitrogen inputs and also responds to applied fertilizer nitrogen. In addition, the lines evaluated have the ability to dilute nitrogen to a great extent. It is concluded that switchgrass is a promising new forage and biomass producer for Florida, especially in present times due to increasing nitrogen fertilizer costs and decreasing availability.

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